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1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE 2006	3. REPORT TYPE AND DATES COVERED Invited Review-Journal of Thermal Biology

4. TITLE AND SUBTITLE
The Thermoregulatory Consequences of Heat Stroke: Are Cytokines Involved?

5. FUNDING NUMBERS

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8. PERFORMING ORGANIZATION REPORT NUMBER
MISC 05-22

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Same as #7 above

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

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14. SUBJECT TERMS
heat stress; hyperthermia; hypothermia; fever; interleukin-1; interleukin-6, tumor necrosis factor, radiotelemetry, gene knockout, sepsis

15. NUMBER OF PAGES
15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unclassified

Invited Review

The thermoregulatory consequences of heat stroke: Are cytokines involved?

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Abstract

The thermoregulatory changes induced by prolonged heat exposure consist of hyperthermia in response to direct heat exposure, and a biphasic response consisting of hypothermia followed by "fever", which develops during long-term recovery. This review discusses the importance of these thermoregulatory responses for prediction of heat stroke morbidity and mortality and the potential role of endogenous cytokines in the regulation of these responses. Current data suggest that the magnitude and duration of hypothermia is directly related to severity of the heat insult, whereas "fever" is a biomarker of the systemic inflammatory response syndrome (SIRS) that ensues during heat stroke recovery. Correlation studies showing elevated cytokine concentrations in human and animal heat stroke models suggest an adverse role of these substances in heat-induced SIRS, although few neutralization studies have been conducted to support this hypothesis. Preliminary results from cytokine and cytokine receptor knockout mice suggest that cytokines may not be involved in the thermoregulatory responses to heat stroke, but in some cases appear to have a protective, permissive role for heat stroke survival.
Published by Elsevier Ltd.

Keywords: Heat stress; Hyperthermia; Hypothermia; Fever; Interleukin-1; Interleukin-6; Tumor necrosis factor; Radiotelemetry; Gene knockout; Sepsis

Contents

1. Introduction

Heat stroke is a life-threatening illness clinically diagnosed as core temperature (T_c) in excess of 41.0 °C,

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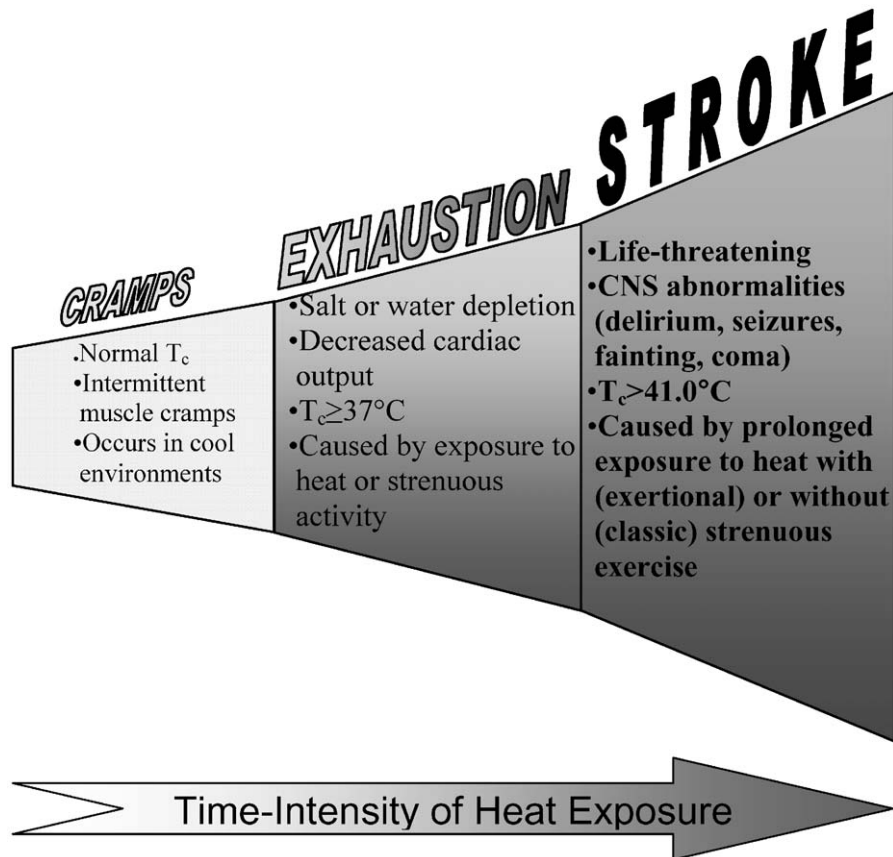E-mail address:* lisa.leon@na.amedd.army.mil.

Fig. 1. The heat illness continuum is best viewed as a continuum with symptoms of increasing severity appearing as one progresses from heat cramps, to heat exhaustion and ultimately heat stroke, the most deleterious condition. Note the specific core temperature (T_c) values associated with heat exhaustion and heat stroke, despite wide T_c variability observed in human and animal studies.

presence of hot, flushed dry skin and central nervous system (CNS) abnormalities, such as delirium, seizures, and coma (Petersdorf, 1994). Heat stroke represents the most serious condition of the heat illness syndrome, which is typically viewed as a continuum of increasing severity (Fig. 1). The use of specific, well-defined symptoms (e.g., a specific T_c value of 41.0 °C) to define heat stroke suggests detailed understanding of the mechanisms mediating the debilitating effects of this syndrome. However, the etiology of the heat stroke syndrome is poorly understood and the mechanisms mediating the adverse consequences of this condition remain unknown. Cooling therapy is currently the most effective strategy for the prevention of heat injury, but despite this treatment, ∼30% of heat stroke survivors incur permanent neurological damage (Dematte et al., 1988). Thus, future research is required to develop more effective preventive and treatment strategies.

The thermoregulatory changes induced by prolonged heat exposure consist of two main responses: (1) an immediate hyperthermic response to direct heat exposure, and (2) a biphasic response consisting of hypothermia followed by "fever", which develops during long-term recovery. This review discusses the importance of these thermoregulatory responses as predictors of heat stroke morbidity and mortality. Data showing altered heat stress

responses in gene knockout mice are presented as they relate to a putative role for endogenous cytokines in the regulation/modulation of heat-induced T_c responses and outcome.

2. Cytokines and heat stroke

In the late 1980s to early 1990s, several studies were conducted during the annual pilgrimage to Mecca (the Hajj) in an effort to characterize peripheral cytokine disturbances in heat stroke patients. Several studies at the Hajj determined circulating cytokine concentrations at the time of clinical presentation and following cooling therapy. At the time of admission, elevations in circulating concentrations of interleukin (IL)-1α, IL-1β, IL-1 receptor antagonist (IL-1ra), IL-6, soluble IL-6 receptor (sIL-6R), IL-10, interferon (IFN)γ, tumor necrosis factor (TNF)α, and soluble TNF receptors (sTNFR60 and sTNFR80) are observed (Bouchama et al., 1991, 1993, 2000; Hammami et al., 1997; Hashim et al., 1997). In some cases, only 30–40% of patients show increased concentration of a particular cytokine (e.g., IL-1β and IL-10; Bouchama et al., 1993, 2000), whereas other cytokines, such as IL-6, are significantly elevated in 100% of patients (Bouchama et al., 1993). IL-6 shows the highest correlation with mortality

and neurological symptoms, implicating it as a potential therapeutic target for heat stroke prevention/treatment strategies (Bouchama et al., 1993; Hammami et al., 1997; Hashim et al., 1997). Although attempts to correlate IL-6 with T_c at admission have been unsuccessful, this may be a consequence of variability in presentation times and a wide T_c range between patients. Thus, a role for IL-6 in the regulation of T_c during the heat stroke syndrome has not been demonstrated.

Soluble IL-6R, sTNFR60 and sTNFR80 concentrations are significantly elevated at 24 h post-cooling in one study (Hammami et al., 1997). It is currently unclear if cooling is a consequence of soluble receptor function (i.e., inhibition of endogenous cytokine actions) in this study. An important aspect of cytokine analysis in heat stroke research is to understand the relationship of endogenous levels of a cytokine to its soluble receptor (or natural antagonists), such as exists for TNF and IL-6. In the study by Hammami et al. (1997), TNFα and β concentrations were undetectable at time of clinical admission, whereas sTNFR60 and sTNFR80 concentrations were significantly elevated above controls. The inability to detect circulating TNF concentrations may be the result of localized production (not detectable in serum samples) or the neutralizing activity of the sTNFRs, the latter of which may interfere with assay detection of the cytokine. Interestingly, heat stroke survivors had higher sTNFR concentrations than non-survivors, suggesting a potential detrimental effect of TNF in this syndrome; however, the small sample size ($N = 3$) in this study precludes a definitive conclusion as to the role of these receptors and endogenous TNF in human heat stroke mortality. As will be discussed in more detail at the end of this review, studies from TNF receptor knockout mice suggest that endogenous TNF has beneficial permissive actions for heat stroke survival.

Animal experimentation has typically relied on passive, rather than exertional heat exposure to study the role of cytokines (Adolph, 1947; Gathiram et al., 1987; Hall et al., 2001; Hubbard et al., 1976, 1977; Lord et al., 1984; Romanovsky and Blatteis, 1996; Wilkinson et al., 1988; Wright, 1976; Wright et al., 1977). Elevated circulating concentrations of IL-1, IL-6, IL-8, IL-10, TNF and granulocyte colony stimulating factor have been observed following localized or whole body hyperthermia in primates (Bouchama et al., 2005), rabbits (Lin et al., 1994), mice (Neville and Sauder, 1988; Leon et al., in press) and rats (Chiu et al., 1995, 1996; Haveman et al., 1996; Lin et al., 1997; Liu et al., 2000). As shown with human exertional heat stroke, IL-6 is correlated with heat stroke severity in a primate model of passive heat stroke (Bouchama et al., 2005). Similarities in cytokine responses between passive (animal) and exertional (human) cases of heat stroke indicate the appropriateness of animal models for the study of heat stroke responses, although exertional heat stroke models need to be developed.

Several attempts have been made to correlate cytokine changes with different aspects of the heat stroke syndrome, such as hyperthermia and heat severity. It is typically difficult to find a strong correlation between cytokine levels and T_c in human heat stroke cases, due to differences in clinical treatment strategies and presentation times. For example, weak correlations between reported T_c and cytokine values are common as patients are treated with different cooling regimens and durations (Bouchama et al., 1993, 2000; Hammami et al., 1997; Hashim et al., 1997; Sonna et al., 2004). However, in one study the ability to cool patients from 40 to 38 °C was dependent on the serum level of IL-1β (Chang, 1993). This is the only report implicating endogenous IL-1β in the control of T_c responses in heat stroke patients. This seems rather surprising since several of the cytokines implicated in heat stroke pathophysiology are known regulators of T_c in health and disease (Kluger, 1991; Leon, 2004). On the other hand, lack of correlation between cytokine levels and T_c may be due to tissue rather than circulating concentrations being important in the mediation of the responses. It is anticipated that the application of transgenic, genomic and/or proteomic technologies to the study of heat stroke responses will aid in our understanding of tissue-specific cytokine changes and its relation to the morbidity and mortality of this syndrome.

The anterior hypothalamus is thought to be the main integration site of afferent sensory information for T_c homeostasis. Lin et al. (1994) reported increased plasma and hypothalamic IL-1β levels in heat stroked rabbits, which to my knowledge is the only study to determine cytokine concentrations at a particular tissue site in an experimental model of heat stroke. While systemic injection of the IL-1ra (a naturally occurring antagonist of endogenous IL-1 actions) attenuated the rectal temperature response to heat exposure, the effectiveness of this treatment following administration directly into the hypothalamus was not tested (Lin et al., 1994). In addition, it is unclear if attenuation of hyperthermia was an indirect response following a reduction in cardiovascular strain or vice versa; thus, the specific role of endogenous IL-1β (and other cytokines) in T_c changes induced by heat exposure and/or recovery remains unknown. Given the large volume of data implicating a variety of cytokines in T_c control and heat stroke pathophysiology, it is surprising that more experimental data have not been provided in this area.

3. The hyperthermic response to heat exposure

An elevation of T_c above 41.0 °C (often referred to as fever or hyperpyrexia) is the most widely recognized heat stroke symptom. The basis for a specific T_c cut-off value of 41.0 °C is not readily apparent, but may reflect an attempt to dissociate the degree of hyperthermia observed in heat stroke from that of infection, in which fevers rarely exceed 41.0 °C (Dubois, 1949).

T_c varies dramatically in heat stroke patients with ranges of 41–42 °C commonly observed, but values as high as ~47 °C reported (Bouchama et al., 1993; Chang, 1993; Hammami et al., 1997; Hashim et al., 1997; Lu et al., 2004; Sonna et al., 2004). Austin and Berry (1956) reported T_c values ranging from 38.5 °C to 44.0 °C in heat stroke patients, with 10% of the mortalities occurring below 41.1 °C. Thus, in this study many patients did not meet the clinical T_c criterion of heat stroke. Large variability in T_c values may be due to several factors, including: (1) differences in the time of clinical presentation such that patients' temperature is obtained at varying stages of heat stroke progression and treatment (i.e., hyperthermia vs. cooling), (2) individual differences in the critical thermal maximum (CTM) associated with heat stroke collapse and/ or mortality, and (3) the site of the temperature measurement. Predisposing factors such as medications, infections and cardiovascular disease may enhance susceptibility to heat stroke, which might manifest as a lower CTM prior to collapse.

CTM is defined as the minimum T_c that is lethal to an organism (Cowles and Bogert, 1944; Hutchison, 1961). Wide CTM values are reported for monkeys (~44.5 °C; Gathiram et al., 1987), dogs (37.7–41.1 °C; Drobatz and Macintire, 1996), sheep (43.7–44.0 °C; Hales et al., 1987), rats (40.4–45.4 °C; DuBose et al., 1983; Hubbard et al., 1976; Lord et al., 1984; Ohara et al., 1975; Wright et al., 1977), mice (42.7 °C and ~44–45 °C; Leon et al., 2005; Wright, 1976), and salamanders (~33 °C; Hutchison and Murphy, 1985). Adolph (1947) determined the CTM in cats (~43.5 °C), dogs (41.7 °C), and rats (42.5 °C) and proposed differences in tissue susceptibility as responsible for CTM variability between species, although tissue injury was not measured in this study. Similarly, Malamud et al. (1946) proposed direct thermal injury to the thermoregulatory centers of the brain as the primary mechanism of mortality, despite an inability to detect hypothalamic injury at autopsy of 125 fatal cases of heat stroke. The ability to induce T_c of 41.6–42.0 °C in humans (within the reported CTM range) with no adverse clinical effects illustrates the inability to rely on a specific CTM for tissue injury predictions (Bynum et al., 1978). Similarly, rectal temperature of 41.9 °C has been recorded in competitive runners showing no adverse clinical signs of heat injury, suggesting that this level of elevated T_c is tolerable in humans (Maron et al., 1977). Of course, runners may be acclimatized to elevated T_c due to repeated exposure during intensive training regimens.

Methodological inconsistencies between studies may account for variability of CTM values. One of the most dramatic differences between studies is the ambient temperature (T_a) used to induce heat stroke. The T_a used in animal studies ranges from 38.6–59.4 °C, making study comparisons difficult (Adolph, 1947; DuBose et al., 1983; Gathiram et al., 1987; Hubbard et al., 1977; Leon et al., 2005; Ohara et al., 1975; Wright, 1976, 1977). The physiological relevance of 59.4 °C is questionable since

this T_a is not routinely encountered in nature (Adolph, 1947). Similarly, the majority of these studies exposed animals to pre-heated environmental chambers (Adolph, 1947; DuBose et al., 1983; Gathiram et al., 1987; Heidemann et al., 2000; Hubbard et al., 1977; Ohara et al., 1975; Wright, 1976; Wright et al., 1977), which represents a heat "shock" rather than heat "stress" paradigm (Leon et al., 2005). In vitro studies have also used heat shock paradigms (e.g., 42–43 °C water bath exposure for 1 h) to examine responses in different cell types (D'Souza et al., 1994; Watanabe et al., 1997, 1998). Heat stroke severity is influenced by the rate of core heating such that rapid exposure to a pre-heated chamber may not provide sufficient lag time for the thermoregulatory system to sense and respond to a dramatic shift in T_a before T_c reaches lethal levels (Flanagan et al., 1995; Hutchison, 1961). Thus, the physiological relevance of the heat shock paradigm is unclear.

T_c values of heat stroke patients (and animals) will differ depending on the site of measurement. In humans, esophageal temperature is the most accurate and responsive to changes in blood temperature, although instrumentation may not be feasible in severely injured, unresponsive patients. Rectal temperature has a slower response rate and gives slightly higher readings than esophageal temperature (Bynum et al., 1978). Oral temperature is rapidly measured, but may provide inaccurate (low) readings due to hyperventilation in the heat stroke patient (Cole, 1983). The recent development of remote T_c sensing by radiotelemetry is a powerful technique that is applicable to both human and animal studies of heat stress (Leon et al., 2005; O'Brien et al., 1998). In humans, T_c may vary as the pill is swallowed and travels through the gastrointestinal tract. In animal models, the use of radiotelemetry has significantly improved experimental design by permitting an assessment of rapid and long-term T_c changes in conscious, freely moving animals (Leon et al., 2005). It is anticipated that these advances in T_c monitoring will significantly improve our ability to model heat stroke responses in experimental test species, permitting more detailed analysis of alterations in thermoregulatory mechanisms occurring under heat stroke conditions.

4. Factors that influence the thermoregulatory response

The T_c profile displayed during heat exposure in experimental animals is characterized as a triphasic hyperthermic curve (Fig. 2). Characteristics of this curve include a rapid increase in T_c (from baseline to thermoregulatory equilibrium), establishment of an equilibrium plateau (i.e., a slower T_c rise), and a final rapid progression that ensues, following thermoregulatory breakdown, leading to $T_{c,Max}$ (may or may not represent the CTM). This T_c pattern is predictable and highly reproducible between species, although individual variability exists in the time to reach $T_{c,Max}$ (Leon et al., 2005; Ohara et al., 1975). Ohara et al. (1975) performed segmental analysis of the three
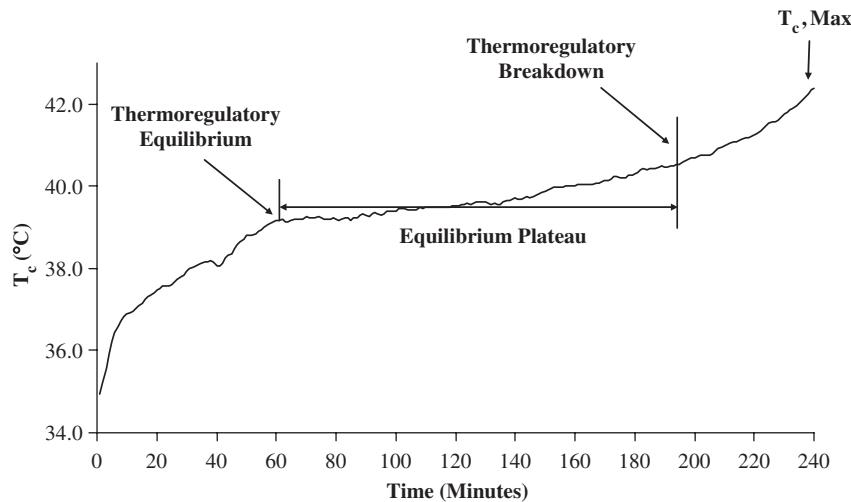
Fig. 2. Core temperature (T_c) data from a male C57BL/6J mouse showing the typical triphasic hyperthermic curve during heat exposure. Ambient temperature during heat exposure was $39.5 \pm 0.2\,^{\circ}$C. T_c were collected at 1-min intervals using the intraperitoneal implantation of a radiotelemetry device. Time 0 represents the start of heat exposure. Adopted from Ohara et al. (1975).

phases of this T_c profile, showing that the greatest inter-individual variability exists in the slope of the equilibrium plateau. The equilibrium plateau represents the period during which physiological and behavioral reflexes are stimulated to enhance heat loss and reduce heat gain. In animals, typical responses include tail vasodilatation to shunt core blood to the skin for dry heat loss (the tail is an important thermoregulatory organ in rodents) and the spreading of saliva and/or urine onto highly vascularized body surfaces to enhance evaporative cooling (rats do not sweat; Elmer and Ohlin, 1970; Hainsworth, 1967; Ohara et al., 1975). High humidity inhibits core cooling as it attenuates evaporation of saliva (or sweat in humans) from the body surface, resulting in enhanced mortality (Furuyama, 1982). In rats, the degree of saliva spreading during the equilibrium plateau is directly related to strain differences in heat tolerance, suggesting a genetic influence on this response (Furuyama, 1982). In humans, racial, geographic and sex differences in heat tolerance have been noted (Carter et al., 2005; Stallones et al., 1957). While it is assumed that differences in acclimatization profiles may account for heat tolerance differences, it is also expected that genetic factors will eventually be identified to account for differences between human populations.

In rodents, hydration level is the most significant factor impacting temperature homeostasis during the equilibrium plateau (Stricker and Hainsworth, 1970). Rats with access to water during heat exposure show increased thermo-tolerance due to behavioral spreading of water (rather than saliva) on the body surface (Stricker and Hainsworth, 1970). Surgical removal or ligation of rat salivary glands sharply elevates T_c and reduces the duration of the plateau (Hainsworth, 1967; Horowitz et al., 1983; Stricker and Hainsworth, 1970). Similarly, dehydration is expected to limit salivary gland secretion and urine volume, thus inhibiting salivary cooling mechanisms. Forty-eight hour

dehydrated rats show a significant reduction in duration of the equilibrium plateau compared to controls (Wright et al., 1977). Interestingly, behavioral spreading of saliva is inhibited in dehydrated rats at non-lethal T_c ($< 40\,^{\circ}$C) as a proposed method of conserving fluid stores until lethal T_c are encountered (Stricker and Hainsworth, 1970). The enhanced release of antidiuretic hormone (ADH) in response to dehydration has been proposed as a mechanism for this response (Junqueira et al., 1967; Stricker and Hainsworth, 1970).

In mammals and poikilotherms, a temporal pattern of heat susceptibility and hydration state related to the circadian cycle has been noted (Hutchison, 1961; Wright et al., 1977). CTM is significantly lower in the afternoon (12–16 h) compared to morning (8–12 h) and evening (16–20 h) in rats (Wright et al., 1977). It is important to note that heat stress experiments are typically performed during the inactive (lights on or daytime) period in rodents (Adolph, 1947; Dubose et al., 1983; Hubbard et al., 1976, 1977; Leon et al., 2005; Lord et al., 1984; Ohara et al., 1975; Romanovsky and Blatteis, 1996; Wright, 1976; Wright et al., 1977). It is not known if T_c responses will differ if heat stress is initiated during the active (lights off or nighttime) period in nocturnal species. Previous data showing a direct correlation between high baseline T_c and mortality (Hubbard et al., 1976) would suggest that heat stroke susceptibility will be elevated during the nocturnal period of rodents (when baseline T_c and activity are elevated), but this hypothesis has not been directly tested. Additionally, a study of rodent heat stress responses during the nocturnal (active) period would more closely simulate the human condition since elevated ambient temperatures are typically encountered during the day when humans are most active.

5. The time-intensity relationship of heat exposure

The fact that the highest mortality rates are observed at least 24 h after the onset of a heat wave suggests that duration and intensity of heat exposure (i.e., the time-intensity relationship) is an important factor to consider for heat injury prediction; it is expected that attempts to limit either of these variables will significantly improve heat stroke outcome (Dematte et al., 1988; Kark et al., 1996; Naughton et al., 2002; Ramlow and Kuller, 1990).

The mathematical concept of thermal area (TA) has been used to evaluate thermal load imposed by heat exposure with post hoc analysis of rodent T_c curves demonstrating a correlation between TA and the morbidity and mortality of heat stroke (Flanagan et al., 1995; Hubbard et al., 1977; Leon et al., 2005). TA (expressed in degree–minutes) represents the area under the T_c curve and is calculated as $\sum\{$(time intervals in minutes) $\times$ (T_c in °C above CTM at the start of the interval $+$ T_c in °C above CTM at the end of the interval)$\}$ (Fig. 3). A CTM value of 40.4 °C has traditionally been used for TA calculations as it represents the lowest T_c at which death has been observed in a rat heat stroke model (Dubose et al., 1983; Flanagan et al., 1995; Hubbard et al., 1976). However, the universal application of this CTM for TA calculations to all species may be inappropriate for several reasons. First, CTM varies with the methodology used to induce a thermal load (e.g., heat shock vs. heat stress paradigms) and shows considerable individual and species variability, as previously described. Second, the CTM value of 40.4 °C was based on an exhaustive heat stroke model in rats, whereas many models and instances of heat stroke are of a passive nature (Chiu et al., 1995, 1996; Haveman et al., 1996; Kao and Lin, 1996; Leon et al., 2005; Lin et al., 1994, 1997; Liu et al., 2000; Romanovsky and Blatteis, 1996; Wilkinson et al., 1988; Wright, 1976; Wright et al., 1977). Third, noted species (Hutchison, 1961), strain (Furuyama, 1982), seasonal (Hoar, 1955; Hutchison, 1961), circadian (Hutchison, 1961; Kosh and Hutchison, 1968; Wright et al., 1977), photoperiod (Hutchison, 1961), geographic (Carter et al., 2005; Hutchison, 1961) and sex effects (Aoki et al., 1998; Carpenter and Nunneley, 1988; Furuyama, 1982; Lublin et al., 1995; Ohara et al., 1975; Mehnert et al., 2002) on heat susceptibility suggest that considerable variability of CTM will exist between studies depending on one or all of these factors. For example, the lowest observed CTM in passively heat stressed mice is 40.7 °C (unpublished observations). Whether this value is species-specific, a condition of the ambient temperature used to induce heat stress (39.5 °C), a strain effect (C57BL/6J) or indicative of a difference in the heating rate between exhaustive and passive heat models is unknown.

The most accurate method for applying TA calculations to heat stroke responses is to determine the CTM for each species and experimental condition under study. Animal models of heat stroke are often confounded by experimental techniques (e.g., restraint and/or anesthesia), which affect T_c homeostasis. Thus, CTM determinations are essential under a variety of experimental conditions to accurately determine the effects of these methodologies versus that of heat exposure on experimental outcome (i.e., mortality). However, ethical concerns regarding the use of mortality as a study endpoint typically prohibits this approach, and it is a time-consuming proposition. As an alternative, it is suggested that TA be calculated for all points at which T_c is greater than the T_a used to impose heat stress (Leon et al., 2005). It is anticipated that this approach will help to standardize TA determinations
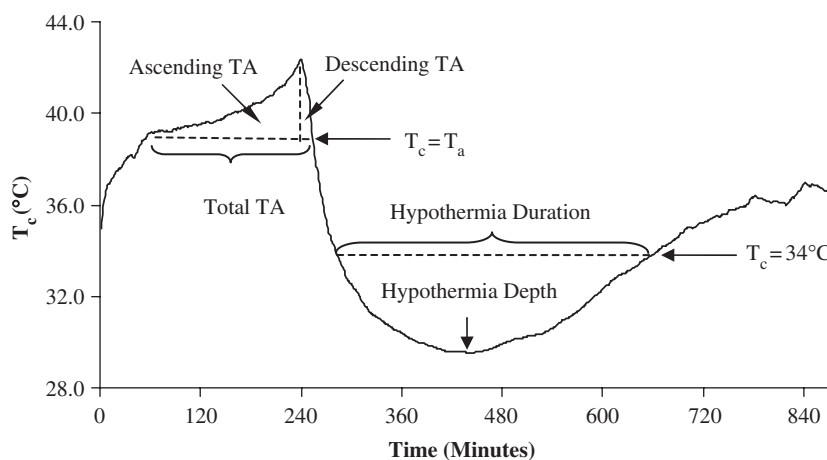

Fig. 3. Typical core temperature (T_c) response of a male C57BL/6J mouse during heat exposure at T_a of 39.5 $\pm$ 0.2 °C (0–240 min) and recovery at T_a of 25 $\pm$ 2 °C (240–840 min). The T_c response during heat exposure is depicted by a triphasic hyperthermic curve and during recovery as hypothermia, whose depth and duration is directly related to heat severity (Leon et al., 2005). Characterization of the triphasic hyperthermic curve is provided by an analysis of thermal load, which is calculated as thermal area (TA; see text for details). Ascending TA assesses thermal load (heat gain) and descending TA assesses cooling (heat loss). TA is calculated for all T_c values that are greater than heat exposure T_a ($T_c = T_a$). Hypothermia duration represents the total time that $T_c < 34.5$ °C, which represents the lowest baseline T_c observed in an undisturbed mouse (Leon et al., 2005). Hyopthermia depth is the lowest 1-min T_c value observed in a heat stressed mouse during recovery. T_c was collected at 1-min intervals using the intraperitoneal implantation of a radiotelemetry device. Time 0 represents the start of heat exposure.

between studies that are using different ambient temperatures for heat exposure. For the T_c curve depicted in Fig. 3, TA was calculated for all time points at which $T_c > T_a$; a correlation between TA and heat severity was detected and supports results from earlier studies in which the CTM of 40.4 °C was used (Hubbard et al., 1976, 1977; Leon et al., 2005).

6. Ambient temperature effects on heat stroke outcome

The breakdown of TA into its ascending and descending aspects allows differences in heat gain (ascending TA) versus heat loss (descending TA) mechanisms to be detected between individuals or animal populations (Fig. 3; Leon et al., 2005). The T_a during heat exposure has a direct impact on ascending TA as the rate of heating is increased with elevations in T_a. Perhaps contrary to expectation, a high rate of heating can induce enhanced mortality despite core temperature remaining elevated above a critical level for a shorter period of time. For example, exercise-induced exhaustion in rats induces greater mortality at a lower $T_{c,Max}$ (~0.4 °C difference) than passive heat exposure (Hubbard et al., 1978). This relationship holds true even under conditions in which TA is maintained constant between exhaustive and passive heat stress groups, indicating a direct effect of the rate of heat gain (enhanced under exercise conditions) on thermal injury. The rate of heating has been shown to have a direct impact on hyperthermic cytotoxicity (Herman et al., 1981; Hubbard et al., 1978), tissue damage, as assessed by serum levels of creatine kinase, lactate dehydrogenase and aspartate aminotransferase (Manjoo et al., 1985), and heat shock protein expression (Flanagan et al., 1995).

The development of heat mitigation techniques to enhance core cooling is based on the premise that the rate of heat loss has the largest impact on heat stroke outcome (Wyndham, 1966). The impact of recovery T_a (which affects cooling rate) on heat stroke mortality may be assessed by a determination of the descending TA or rate of heat loss (Fig. 3). Due to the large surface area to body mass ratio (SA:M_b) of small rodents, T_a has a significant impact on the rate of heat exchange with the environment. In mice, heat stroke survival is significantly enhanced with decreases in recovery T_a (Leon et al., 2005; Wilkinson et al., 1988; Wright, 1976). The thermoneutral zone (TNZ; corresponds to minimal metabolic rate) of rats is 25–28 °C whereas mice prefer T_a ranges of 30–35 °C (Gordon, 1993; Leon, 2005). As expected from the curve shown in Fig. 3, an enhanced rate of core cooling during recovery at a cool T_a (i.e., below the TNZ) significantly reduced descending TA and enhanced survival (Leon et al., 2005). Mice heat stressed to the CTM of 42.7 °C showed an increase in descending TA and mortality during recovery at T_a of 30 °C compared to 25 °C (100 vs. 8% mortality, respectively; Leon et al., 2005). As might be expected, the cooling rate of mice housed at 30 °C was significantly greater (0.10 ± 0.01 °C) than that at 25 °C (0.06 ± 0.01 °C; ANO-

VA, $P = 0.002$). Similarly, previous studies in humans have indicated that decreasing T_c to < 39.8 °C within 30 min of presentation significantly decreases mortality (Dematte et al., 1988).

There are several pre-disposing factors (e.g., cardiovascular deficiency, diuretics use) that affect thermotolerance in animal and human populations. In many cases, these factors have been shown to have a direct impact on the thermoregulatory response to direct heat exposure, but their effects on the pathophysiological responses during recovery remain unknown. It is hypothesized that thermoregulatory efficiency during heat exposure and recovery will have the most immediate impact on heat stroke recovery. However, despite appropriate behavioral and physiological adjustments, organisms will continue to collapse from heat exposure. Thus, advances in treatment strategies will be required to limit heat stroke mortality during the hours, days, weeks, and months of recovery. It is anticipated that an understanding of the thermoregulatory changes occurring during recovery will shed light on the physiologic condition of heat stroke victims and provide important feedback in terms of treatment efficacy and re-establishment of homeostasis. Discussion is provided below on the usefulness of T_c recovery changes as biomarkers of heat stroke severity.

7. Hypothermia as a biomarker of heat severity

While the triphasic hyperthermic response to heat exposure is well-defined, the T_c response displayed during long-term (i.e., > 24 h) recovery has received less attention. This is rather surprising since the magnitude, duration and direction of T_c changes displayed during recovery may provide information regarding severity and etiology of the initial heat insult. In experimental animals, hypothermia is the predominant heat stress recovery response (Fig. 3). Heat-induced hypothermia is the term used to define the seemingly paradoxical decrease of T_c below baseline levels during recovery (Romanovsky and Blatteis, 1996). Although the depth and duration of hypothermia varies between studies, heat-induced hypothermia has been observed in cats (Adolph, 1947), guinea pigs (Adolph, 1947; Romanovsky and Blatteis, 1996), mice (Leon et al., 2005; Wilkinson et al., 1988; Wright, 1976), rats (Lord et al., 1984) and salamanders (Hutchison and Murphy, 1985). Regardless of the experimental conditions, hypothermia of > 1.0 °C is commonly observed. In some cases, hypothermia is quite profound such that T_c is regulated only a few degrees above T_a (Leon et al., 2005; Wilkinson et al., 1988; Wright, 1976). In mice, the depth (~1.0–5.0 °C) and duration (~1–24 h) of hypothermia is directly related to severity of the heat insult (Leon et al., 2005; Wilkinson et al., 1988). Thus, the characteristics of the hypothermic response, as depicted in Fig. 3, serve as powerful biomarkers of heat morbidity and mortality. For example, a comparison of individual responses to heat stress indicates that animals experiencing the longest duration

of heat exposure show the largest depth and duration of heat-induced hypothermia during recovery (Leon et al., 2005). Furthermore, post-hoc analysis of mouse T_c curves indicates that mice that are unable to transition out of hypothermia (i.e., re-warm) within 765 min of recovery do not survive (Leon et al., 2005). If a similar relationship exists between heat severity and hypothermic characteristics for humans, this could have important implications for the timing of clinical treatment strategies. However, to my knowledge, hypothermia has not been reported in human heat stroke cases, which may be due to body scaling issues (significantly smaller $SA:M_b$ compared to small rodents) or clinical interventions that have masked the response.

As previously described, recovery T_a can have a significant impact on cooling rate and heat susceptibility. In mice, heat stress recovery at T_a of 30 °C (within the TNZ), which prevents hypothermia development, is associated with enhanced intestinal damage and mortality within ~2 h of recovery (Leon et al., 2005; Wilkinson et al., 1988). This response is correlated with significantly greater descending TA in mice that recovered at T_a of 30 °C compared to 25 °C (Leon et al., 2005). Thus, heat stroke survival in small rodents is dependent on recovery T_a, which in this case appears to be due to effects on the rate of core cooling. A correlation between hypothermia development and prevention of tissue injury suggests that cooling of heat stroke patients to a hypothermic level (i.e., T_c < 37 °C) may be beneficial. Further support for this contention is provided by the use of induced hypothermia, in which T_c is physically decreased using cooling blankets or other methods, as a protective measure during cardiopulmonary bypass surgery and as treatment for cerebral ischemia and stroke (Dietrich and Kuluz, 2003; Marion et al., 1997). To the best of my knowledge, the effect of induced hypothermia on heat stroke outcome has not been tested. The realization that hypothermic treatment would be more efficacious if regulated, rather than forced reductions in T_c were implemented suggests that further studies are required to determine the regulated nature of hypothermia under injurious conditions (Gordon, 2001). Furthermore, if cytokines are regulators/modulators of heat-induced hypothermia or their production is influenced by hypothermia, as previously described for bacterial infections (Arons et al., 1999; Fairchield et al., 2004), they may represent once class of substances that could be targeted to induce hypothermia in a regulated fashion and minimize tissue injury in heat stroke patients.

An intriguing question is whether the mechanism(s) of heat-induced hypothermia is active or passive in nature. There is controversy regarding the regulated nature of heat-induced hypothermia; it is unclear if this T_c response represents a survival mechanism aimed at reducing metabolic demands or an unregulated response due to thermal injury of critical tissues involved in T_c homeostasis. Hypothermia may be beneficial due to a reduction of free radical production at low T_c. Based on the Q_{10}

effect (i.e., the factor by which biochemical reaction rate is changed for each 10 °C change in temperature), one predicts that for each 1 °C decrease in T_c, there will be a ~10% decrease in tissue metabolic requirements (and a subsequent inhibition of the production of harmful tissue end products). The widespread occurrence of regulated hypothermia argues in favor of its function as a survival mechanism.

The first studies to examine the regulated nature of heat-induced hypothermia were conducted by Hutchison and Murphy (1985) in salamanders, which are reliant on the behavioral selection of T_a to regulate T_c. Following heat exposure to the CTM or sub-CTM, heat stressed salamanders selected warmer ambient temperatures compared to controls, which was hypothesized to be a direct result of thermal damage to the hypothalamic controlling centers (Hutchison and Murphy, 1985). The selection of cooler temperatures during the three remaining days of recovery was interpreted as evidence of either (1) a disruption of homeostatic processes due to heat injury to CNS centers or (2) an adaptive thermoregulatory survival mechanism. Unfortunately, a heat clamp experiment, in which the mortality rate of heat stressed salamanders was assessed in a thermal gradient that prevented the selection of cooler ambient temperatures, was not used to directly test these hypotheses.

Active mechanisms of hypothermia development may include reversible metabolic inhibition and/or a decrease in the thermal setpoint. The ability of naltrexone (a wide-spectrum opioid-receptor antagonist) to blunt heat-induced hypothermia in guinea pigs implicates endogenous opioids in the regulation of this response (Romanovsky and Blatteis, 1996). The proposed effect of endogenous opioids is thought to be through the inhibition of metabolism, although this hypothesis has not been directly tested (Romanovsky and Blatteis, 1996). Reversible metabolic depression is a common survival mechanism used by small rodents to diminish metabolic demands under conditions of resource scarcity, tissue injury and infection. Passive mechanisms of hypothermia development may include autonomic failure that results in peripheral vasodilation in the absence of compensatory heat gain, or widening of the inter-threshold zone such that a dead band or poikilothermic type of T_c control is manifest (Romanovsky and Blatteis, 1996). A widening of the inter-threshold zone for thermal effector activation (i.e., T_c becomes dependent on T_a) has been observed during recovery from hyperthermia and bacterial infection (i.e., sepsis) in rodents (Romanovsky and Blatteis, 1996; Romanovsky et al., 1996). It is unclear if this is a universal mechanism of injury-induced hypothermia development or is specific to rodents.

8. "Fever" characteristics are independent of heat severity

Current data suggest that heat stroke pathophysiologic responses are the result of a systemic inflammatory response syndrome (SIRS) that ensues following thermal

injury, rather than a direct effect of heat exposure, per se (Bouchama and Knochel, 2002). In humans, anecdotal evidence suggests that fever is a symptom of heat stroke, persisting for 7–14 days following clinical presentation (Attia et al., 1983; Austin and Berry, 1956; Malamud et al., 1946). The occurrence of this thermoregulatory response at late stages of the heat stroke syndrome suggests that fever, which is a tightly controlled physiologic response to stress, is more directly related to the complications ensuing after heat exposure, than to the initial heat insult. Although "fever" is reported immediately upon admission in many clinical heat stroke cases, this likely represents the hyperthermic response to direct heat exposure rather than a true fever. Fever is defined as a regulated increase in the hypothalamic thermal setpoint and is observed in response to several stimuli including bacterial infection, stress and tissue inflammation (Kluger, 1991). Fever results from the coordinated action of behavioral and physiological mechanisms that increase heat production and decrease heat loss to raise T_c to a new elevated level. The presence of tissue injury (Bouchama et al., 2005; Dematte et al., 1988; Lu et al., 2004; Malamud et al., 1946), cytokines (Bouchama et al., 1991, 1993, 2005) and endotoxemia (Graber et al., 1971) in heat stroke suggests that the persistence of fever beyond the initial day of heat exposure (and clinical admission) may be a result of heat-induced SIRS. This long-term occurrence of fever may also account for it not being widely recognized as a heat stroke recovery response in animal studies. Due to reliance on rectal probes, restraint and/or anesthesia for T_c measurements in animals, thermoregulatory responses to heat stress have typically not been examined across multiple circadian cycles (Bouchama et al., 2005; Lin et al., 1994, 1997). The advent of radiotelemetry has overcome this experimental limitation and shown that a "fever-like" elevation is observed from ~24–36 h following heat exposure in mice (Leon et al., 2005). Thus, mice develop a biphasic thermoregulatory response during heat stress recovery that consists of an initial, profound hypothermia (~6–7 °C below baseline) followed by a fever-like elevation the following day (Leon et al., 2005). Interestingly, this biphasic T_c response is remarkably similar to that observed in response to sepsis (Fig. 4), suggesting that endotoxemia may be one of several stimuli inducing this response; elevated endotoxin levels have been reported in human and animal models of heat stroke (Bouchama et al., 1991; Gathiram et al., 1987). Evidence in favor of the heat-induced T_c elevation being a true fever, similar to that observed during sepsis, include the observations that it is displayed only during the day (inactive period; Leon et al., 2005) and is associated with elevated plasma levels of IL-6 (Leon et al., submitted), a recognized endogenous pyrogen (Kluger, 1991).

Mice show virtually identical increases in the fever-like response (~1.0–1.5 °C) irrespective of heat severity (Leon et al., 2005). This is in contrast to hypothermia, which is directly related to heat severity in mice, as previously
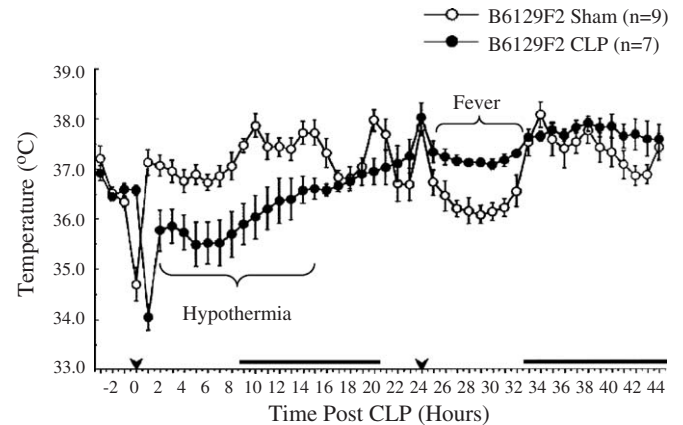

Fig. 4. Typical core temperature (T_c) response observed in male mice during 48 h of recovery from cecal ligation and puncture (CLP; sepsis). This biphasic T_c response is similar in characteristics (hypothermia preceding fever) and time course to that observed during heat stroke recovery. T_c was collected at 5-min intervals using the intraperitoneal implantation of a radiotelemetry device; data are presented as 1 h averages for ease of presentation. Black horizontal bars represent lights-off period on a 12:12 h L:D cycle (lights on at 0700 h). Arrow at time 0 indicates time of CLP and at time 24 h represents time of weighing for BW measurement (data not shown).

described (Leon et al., 2005; Wilkinson, et al., 1988). Thus, in contrast to hypothermia, heat-induced "fever" is not a reliable biomarker of heat severity. Interestingly, mice that do not recover from hypothermia to develop "fever", succumb to heat stroke (Leon et al., 2005). It is unclear if this is indicative of a protective function of fever in the heat syndrome or a debilitating effect of prolonged hypothermia. It would be of interest to administer antipyretic drugs, such as NSAIDS, in mice to determine the regulated nature of this fever-like phase and determine its importance for survival.

9. Altered heat stress responses in gene knockout mice

The study of cytokine-mediated heat stroke responses has been limited by technical difficulties, such as a lack of commercial availability of cytokine antibodies and/or antagonists. The recent advent of gene knockout technology provides a technique by which the efficacy of cytokine neutralization on heat stroke outcome measures can be examined in vivo. Gene knockout mice are genetically engineered to lack a functional gene in every tissue of the body and essentially function as "chronic protein neutralization systems" (Sigmund, 1993). There is wide commercial availability of cytokine and cytokine receptor knockout mice, many of which have been used for the study of infectious and inflammatory syndromes. While the development of functional redundancy is an important concern with the use of these models in physiological research (i.e., the redundant/pleiotropic properties of cytokines may allow developmental redundancy to compensate for a missing gene's action), these models also provide several methodological advantages over traditional

techniques (reviewed in Leon, 2005). As will be described in more detail below, my laboratory recently characterized heat stroke responses in cytokine and cytokine receptor knockout mice and showed altered heat stress responses that were unexpected based on previous data that had been provided from correlation studies.

As previously described, high IL-6 levels correlate with heat stroke morbidity and mortality in patients and animal models, yet protective effects of IL-6 neutralization have not been experimentally verified (Bouchama et al., 1993, 2005; Hammami et al., 1997; Hashim et al., 1997). Using a

previously established model in conscious unrestrained mice, my laboratory examined T_c responses and survival rates of IL-6 knockout and wild-type mice following heat exposure to the CTM of 42.7 °C (induces ∼8% mortality; Leon et al., 2005). Fig. 5 shows the 48 h biphasic T_c profile of wild-type and IL-6 knockout mice during heat exposure and recovery at T_a of 25 °C. To assess the function of endogenous IL-6 in the thermoregulatory response to heat exposure, TA and hypothermic responses were characterized, as described in previous sections of this review. Total and ascending TA did not differ between wild-type and IL-
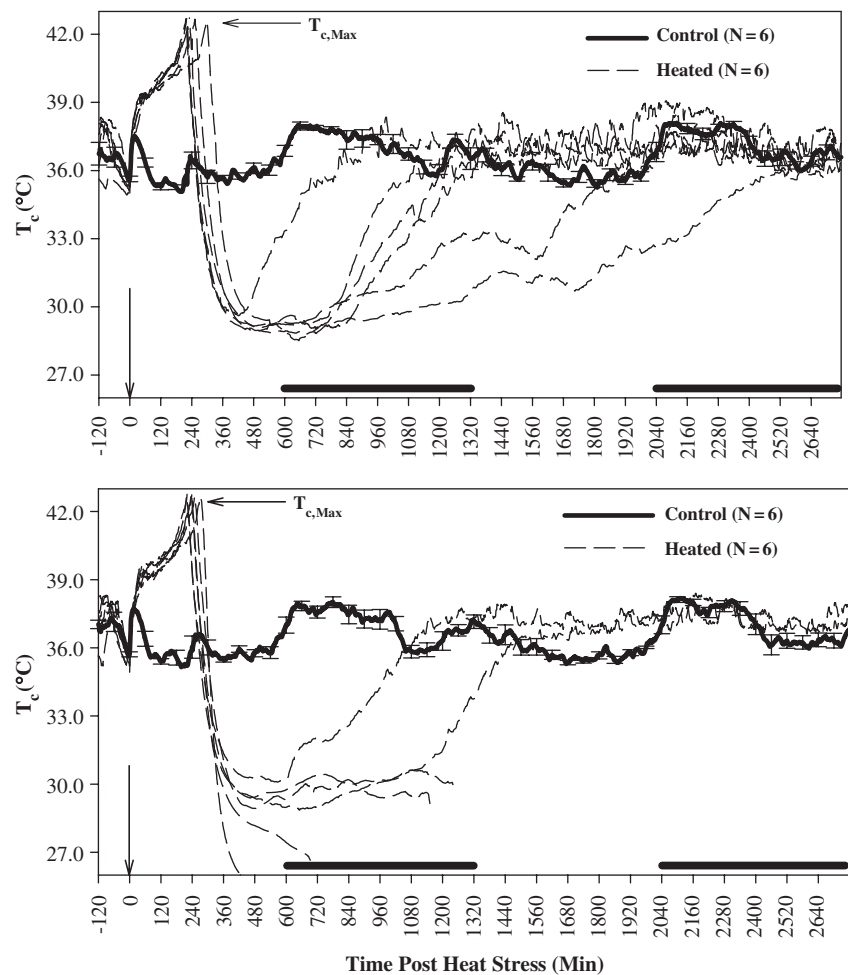
	C57BL/6J (N = 6)	IL-6 KO (N = 6)	P-value
Thermal Area (°C·Min)	275.9±17.3	258.8±8.6	0.424
Ascending TA	242.1±17.3	217.3±7.8	0.220
Descending TA	33.8±2.5	41.5±1.8	0.034
Hypothermia Depth (°C)	29.3±0.1	29.4±0.3	0.752
Survival (%)	100	33	0.025

Fig. 5. 48 h core temperature (T_c) response of male C57BL/6J (wild-type; top) and IL-6 knockout (bottom) mice during heat exposure ($T_a = 39.5 ± 0.2$ °C) to a maximum T_c ($T_{c,Max}$) of 42.7 °C and recovery at T_a of 25 ± 2 °C. Note that IL-6 knockout mice had significantly greater descending TA (slower cooling rate) and enhanced mortality compared to wild-type mice. Hypothermia depth did not differ between groups. Sample sizes are indicated in parentheses. T_c was collected at 1-min intervals using the intraperitoneal implantation of a radiotelemetry device. Arrow at time 0 represents start of heat exposure. Black horizontal bars represent lights-off period on a 12:12 h L:D cycle (lights on at 0700 h).

6 knockout mice, indicating that thermoregulatory mechanisms to direct heat exposure were not altered in the absence of IL-6 actions. This was not a particularly surprising finding since increased circulating IL-6 levels were not detectable in heat stressed mice at $T_{c,Max}$ (Leon et al., in press). However, analysis of the cooling phase of recovery indicated that IL-6 knockout mice had a significantly greater descending TA than their wild-type controls (Fig. 5; Student's *t*-test, $P = 0.034$). Thus, IL-6 knockout mice did not dissipate core heat to the environment as effectively as wild-type mice during recovery (it is important to note that body weight did not differ between genotypes). It is hypothesized that differences in cooling rate were responsible for *enhanced* mortality in the IL-6 knockout compared to wild-type mice within 24 h of recovery (see Table associated with Fig. 5; 33 vs. 100%, respectively). While these data do not refute the hypothesis that elevated IL-6 levels are detrimental to heat stroke survival (as suggested by correlation studies), they are suggestive of a permissive effect of IL-6 that is required for heat stroke recovery. Similar results are reported for IL-6 knockout mice in the sepsis syndrome (Leon et al., 1998). Note that hypothermia depth was virtually identical between wild-type and IL-6 knockout mice (29.3 ± 0.1 vs. $29.4 \pm 0.3\,°C$, respectively) suggesting a lack of involvement of endogenous IL-6 in the regulation of this T_c response. Similarly, although only 2 animals survived through 48 h of observation, the fever-like response from ~24–36 h appeared to remain intact in IL-6 knockout survivors; thus, IL-6 may not be regulating/modulating this T_c response either, although a larger sample size is required to statistically verify this conclusion. It would be interesting to conduct a feedback experiment in which IL-6 is administered to knockout mice during different stages of heat stroke recovery to determine (1) the endogenous level of IL-6 that is required for survival (i.e., baseline or elevated levels), and (2) the time point during recovery at which IL-6 actions are beneficial. Interestingly, the contradiction between the results from IL-6 knockout mice and those reported in correlation studies suggest that IL-6 may have both pro- and anti-inflammatory properties, which are dependent on the cytokine milieu in which it functions. Thus, future studies should be directed at determining changes in circulating levels of other cytokines influenced by heat exposure and IL-6 to determine how the absence of this cytokine has altered the balance of others mediators in survivors and non-survivors of this syndrome.
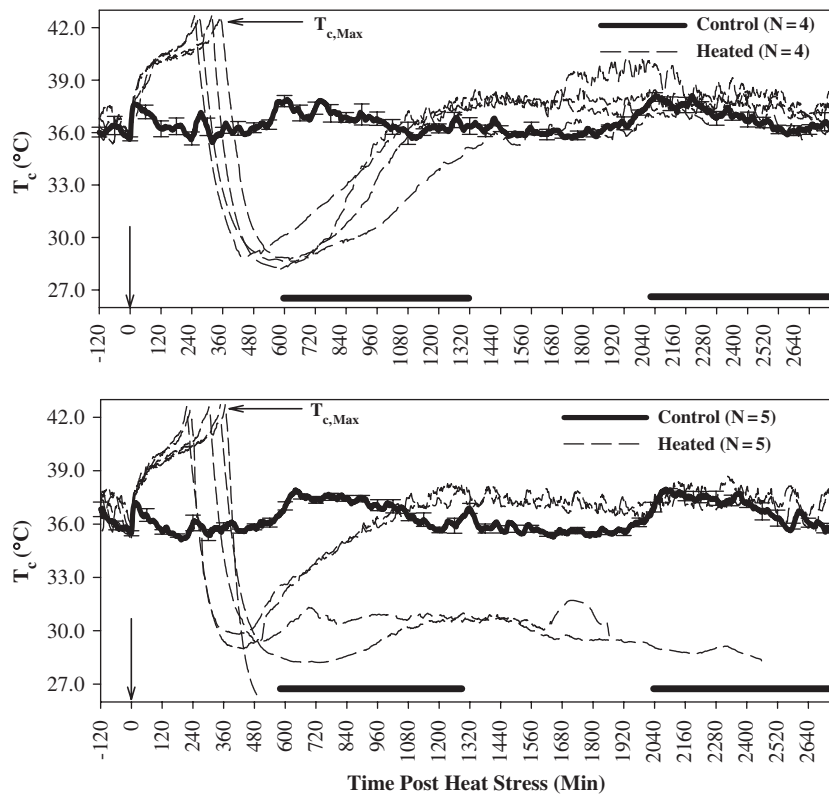
Similar to that observed for IL-6, preliminary data from TNF p55/p75 receptor (TNFR) knockout mice also contradict findings obtained from correlation studies. High TNF levels in patients are suggestive of an adverse role of this cytokine in the heat stroke syndrome (Hammami et al., 1997). Thus, one might hypothesize that TNFR knockout mice (mice that produce endogenous TNF, but are unable to respond to the cytokine due to an absence of the signaling receptors) would show reduced morbidity and mortality to heat stress compared to wild-type controls.

Fig. 6 shows the 48 h biphasic thermoregulatory profile of wild-type and TNFR knockout mice following heating to the CTM of $42.7\,°C$ and during recovery at T_a of $25\,°C$. TA analysis showed significantly decreased total, ascending and descending TA in TNFR knockout mice, suggesting that these mice became hyperthermic and cooled more rapidly than their wild-type counterparts (Table associated with Fig. 6; again, body weight was virtually identical between groups). Although wild-type mice showed a tendency towards enhanced hypothermia depth, as would be expected following accruement of a larger total TA, the difference between groups did not reach statistical significance (28.7 ± 0.2 vs. $29.2 \pm 0.3\,°C$, Student's *t*-test, $P = 0.275$). It is unclear if this is a consequence of the small sample sizes or an indication that the characteristics of the hypothermic response are not indicative of thermal load imposed on these genetic models. Survival rates also did not differ between groups, but showed a tendency towards a decrease in the TNFR knockout group (40 vs. 100% survival, respectively; Chi square, $P = 0.100$). Again, of those TNFR knockout mice that survived through 48 h, a fever-like elevation was evident the day following heat exposure, indicating that endogenous TNF may not be involved in the regulation/modulation of this response. Again, a larger sample size is required to statistical verify this conclusion. If differences in survival are noted between genotypes, this would indicate that despite a more efficient thermoregulatory response to heat exposure, the absence of endogenous TNF actions in some way inhibits the ability of these animals to survive direct heat exposure and/or the SIRS syndrome.

Several questions arise from the findings in gene knockout mice. Have the data from correlation studies been overstated with regards to the deleterious role of endogenous cytokines in the heat stroke syndrome? What are the implications of these findings to the human condition—are similar permissive actions required for survival in heat stroke patients? Is this a condition of the gene knockout model or indicative of a dual role for cytokines (high vs. permissive levels) in heat stroke responses? IL-6 and TNF modulate each other's production—are the reciprocal interactions between these cytokines responsible for the altered heat stroke mortality in IL-6 and TNFR knockout models? Until additional studies using several different experimental techniques to examine the effectiveness of cytokine neutralization in the heat stroke condition are conducted, these questions remain unanswered.

10. Conclusions and perspective

The heat illness syndrome is a continuum of increasing severity that is characterized by predictable disturbances of T_c and cytokine homeostasis. Although much is known regarding the role of endogenous cytokines in the regulation of T_c responses to infection, inflammation and injury, there are little or no data examining the role of these substances in heat-induced T_c responses. The lack of data

Fig. 6. 48 h core temperature (T_c) response of male B6129F$_2$ (wild-type; top) and TNFR knockout (bottom) mice during heat exposure ($T_a = 39.5 \pm 0.2\,^\circ$C) to a maximum T_c ($T_{c,Max}$) of 42.7 $^\circ$C and recovery at T_a of $25 \pm 2\,^\circ$C. Note that TNFR knockout mice had significantly lower total, ascending (thermal load) and descending TA (slower cooling rate) compared to wild-type mice. Hypothermia depth and survival did not differ between groups. Sample sizes are indicated in parentheses. T_c was collected at 1-min intervals using the intraperitoneal implantation of a radiotelemetry device. Arrow at time 0 represents start of heat exposure. Black horizontal bars represent lights-off period on a 12:12 h L:D cycle (lights on at 0700 h).

	B6129F$_2$ (N = 4)	TNFR KO (N = 5)	P-value
Thermal Area (°C·Min)	455.8±29.9	327.6±31.8	0.024*
Ascending TA	408.4±2.3	301.3±31.6	0.044*
Descending TA	47.5±6.6	26.3±0.8	0.010*
Hypothermia Depth (°C)	28.7±0.2	29.2±0.3	0.275
Survival (%)	100	40	0.100

in this area is perplexing for several reasons. First, hypothermia and fever represent predictable responses to heat exposure in rodents and this biphasic T_c response resembles that observed in the endotoxemic/sepsis syndrome, for which a role for several cytokines (e.g., IL-1, IL-6, IL-10, TNF) has been strongly implicated. Thus, it is anticipated that much knowledge can be gained from applying our current understanding of endotoxemic pathophysiology to the study of heat stroke. Second, hypothermia appears to be a sensitive biomarker of heat severity. This may have important implications in the clinical setting since (a) details regarding the initial heat insult are rarely known, and (b) induced hypothermia may have currently unrecognized protective effects that could be utilized as a clinical treatment strategy. Clearly, the regulated nature of heat-induced hypothermia and the beneficial effect of the fever-like response need to be determined. Third, the efficacy of cytokine neutralization on heat stroke morbidity and mortality has not been extensively investigated. Currently, IL-1 is the only cytokine whose neutralization has been examined for its benefit in heat stroke outcome, showing a protective effect (Chiu et al., 1995; Liu et al., 2000). Yet, the mechanism(s) of protection remain unknown. How is the thermoregulatory profile altered with this treatment? Is the beneficial effect a direct thermal effect or due to interactions between changes in thermal load and cardiovascular strain? Unfortunately, current data implicating cytokines in heat stroke responses are mainly from correlation studies showing elevated plasma levels in heat stroke patients and experimental animal models. Correlation data fall far short of revealing the mechanisms of cytokine action, as suggested from the results obtained in IL-6 and TNFR knockout mice. Furthermore, cytokine determinations

have typically been performed at end-stage heat stroke, such that the role of these substances in progression and long-term recovery from heat injury is poorly understood. While the thermoregulatory changes induced by heat exposure represent only one physiological aspect of this complex syndrome, T_c is extremely labile to environmental perturbation and is simple to measure, thus providing rapid and powerful information regarding homeostatic balance of the individual. It is anticipated that more detailed understanding of the role of cytokines in the hypothermic and fever-like responses to heat stroke will provide important insight into the role of these substances in the complex etiology of the long-term consequences of this syndrome.

Acknowledgements

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